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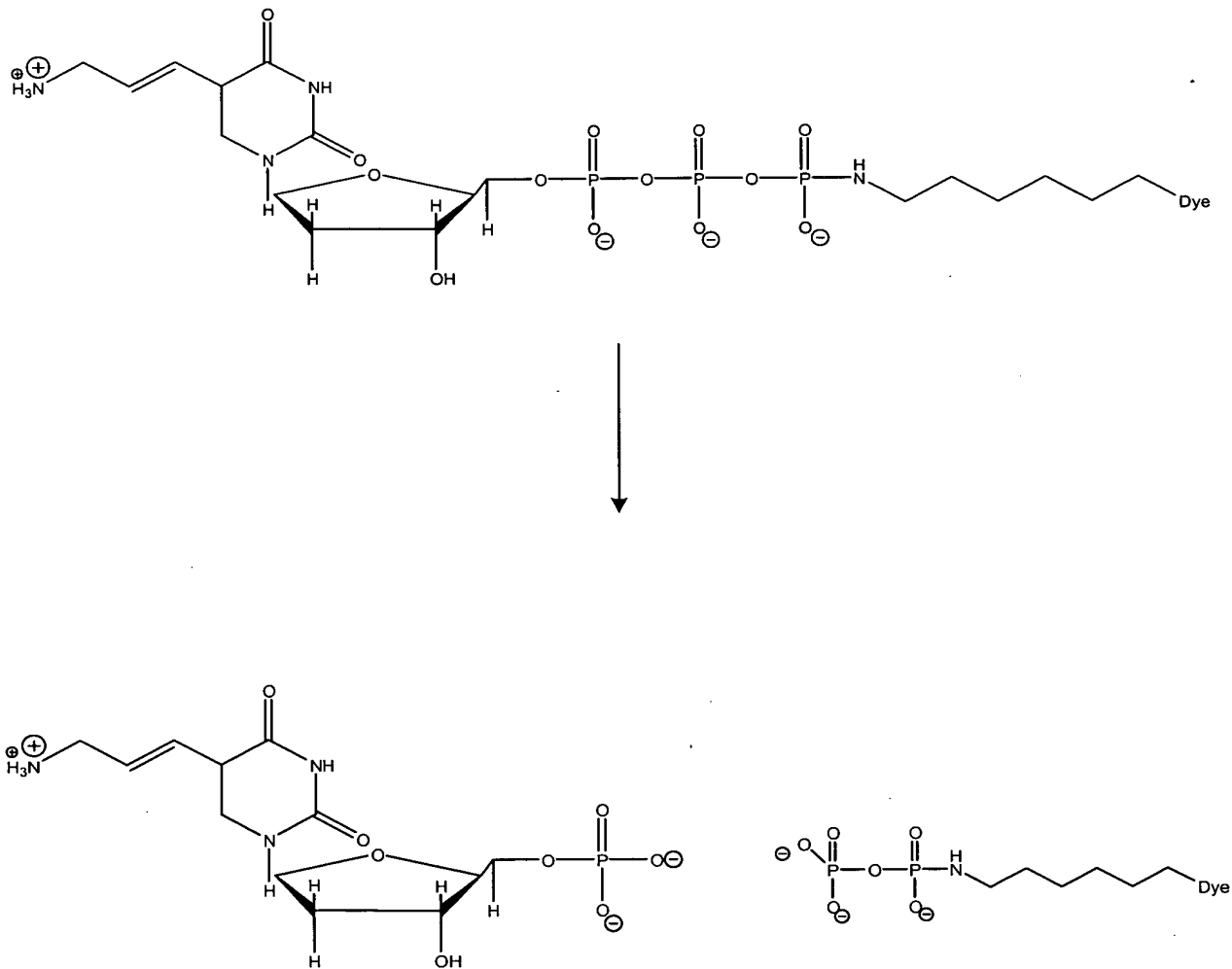


FIG. 1

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NO.	IDEAL CONDITION: ALL BASE AND F ADDUCTS FULLY CHARGED					IN PURE WATER
	Charge on indicated moiety		Net charge			pH 7.0
	NUCLEOBASE	F	NP PROBE	PP-F	Change	Change
1	-3	-3	-9	-6	3	3.25
2	-3	-2	-8	-5	3	3.25
3	-3	-1	-7	-4	3	3.25
4	-3	0	-6	-3	3	3.25
5	-3	1	-5	-2	3	3.25
6	-3	2	-4	-1	3	3.25
7	-3	3	-3	0	3	3.25
8	-2	-3	-8	-6	2	2.26
9	-2	-2	-7	-5	2	2.26
10	-2	-1	-6	-4	2	2.26
11	-2	0	-5	-3	2	2.26
12	-2	1	-4	-2	2	2.26
13	-2	2	-3	-1	2	2.26
14	-2	3	-2	0	2	2.26
15	-1	-3	-7	-6	1	1.26
16	-1	-2	-6	-5	1	1.26
17	-1	-1	-5	-4	1	1.26
18	-1	0	-4	-3	1	1.26
19	-1	1	-3	-2	1	1.26
20	-1	2	-2	-1	1	1.26
21	-1	3	-1	0	1	1.26
22	0	-3	-6	-6	0	0.26
23	0	-2	-5	-5	0	0.26
24	0	-1	-4	-4	0	0.26
25	0	0	-3	-3	0	0.26
26	0	1	-2	-2	0	0.26
27	0	2	-1	-1	0	0.26
28	0	3	0	0	0	0.26
29	1	-3	-5	-6	-1	-0.74
30	1	-2	-4	-5	-1	-0.74
31	1	-1	-3	-4	-1	-0.74
32	1	0	-2	-3	-1	-0.74
33	1	1	-1	-2	-1	-0.74
34	1	2	0	-1	-1	-0.74
35	1	3	1	0	-1	-0.74
36	2	-3	-4	-6	-2	-1.74
37	2	-2	-3	-5	-2	-1.74
38	2	-1	-2	-4	-2	-1.74
39	2	0	-1	-3	-2	-1.74
40	2	1	0	-2	-2	-1.74
41	2	2	1	-1	-2	-1.74
42	2	3	2	0	-2	-1.74
43	3	-3	-3	-6	-3	-2.74
44	3	-2	-2	-5	-3	-2.74
45	3	-1	-1	-4	-3	-2.74
46	3	0	0	-3	-3	-2.74
47	3	1	1	-2	-3	-2.74
48	3	2	2	-1	-3	-2.74
49	3	3	3	0	-3	-2.74

FIG. 2

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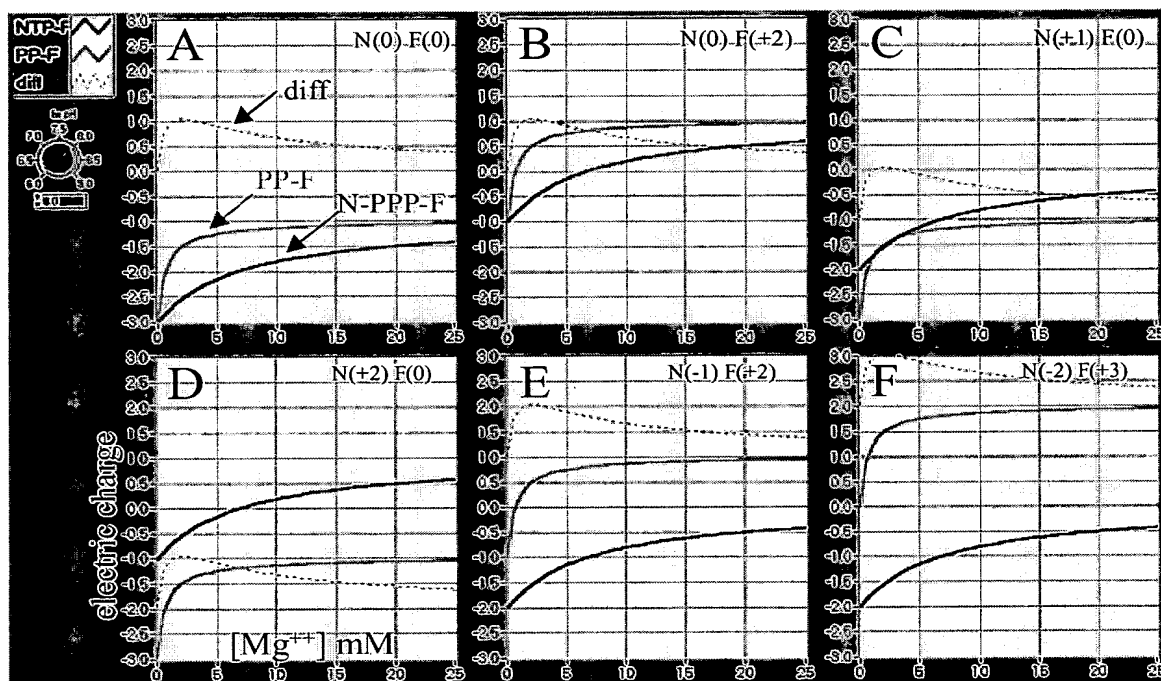
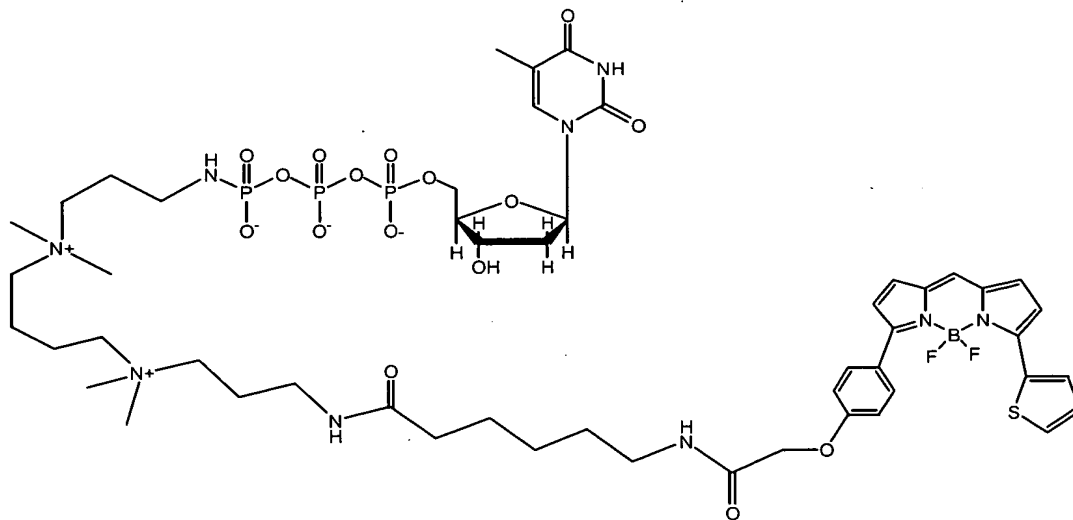


FIG. 3

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dTTP-BQS-BTR

FIG. 4

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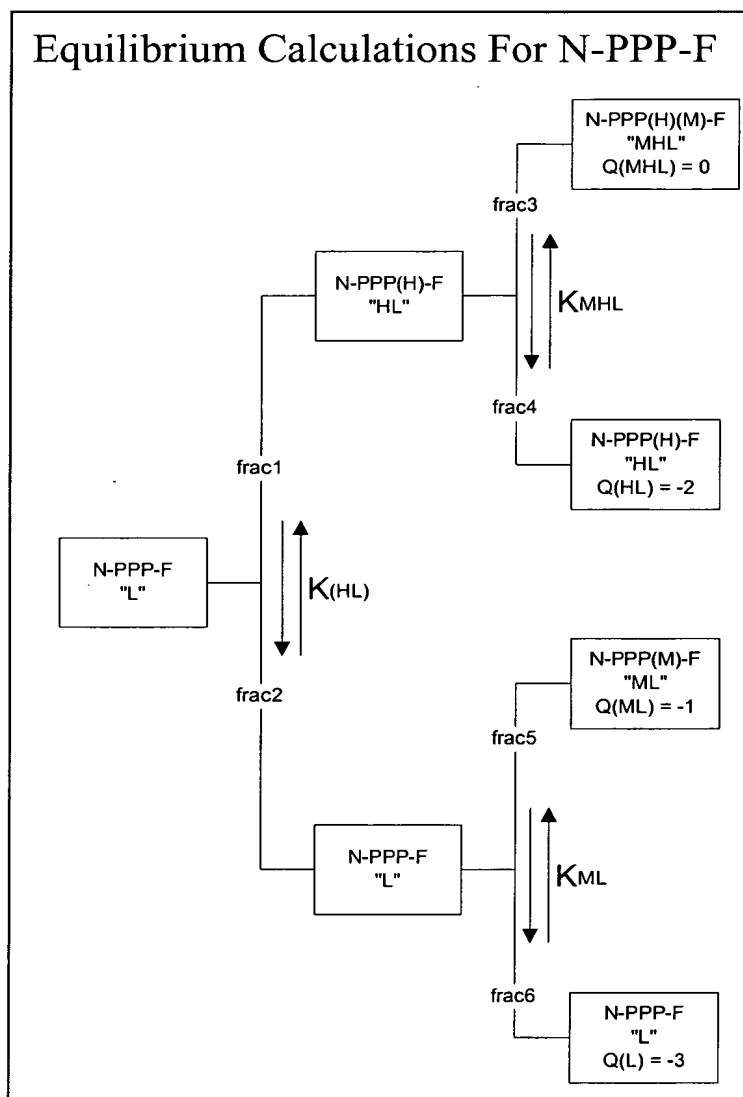


FIG. 5

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COMPOUND	CHARGE	NAME	STRUCTURE
50	N = -2 F = +2	DBA-U-BQS-TAMRA X	
51	N = -2 F = +1	DBA-U-BQS-Oregon 500	
52	N = -1 F = +2	SUC-U-BQS-TAMRA X	

FIG. 6A

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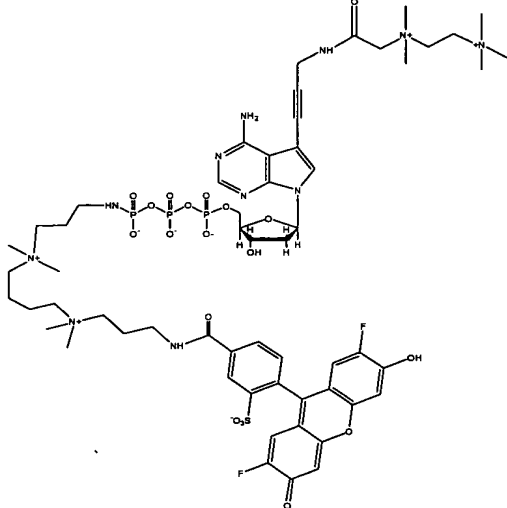
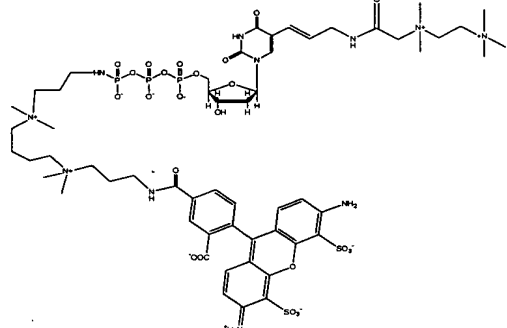
53	N = +2 F = +1	PAA-A-BQS-Oregon 500	
54	N = +2 F = 0	PAA-U-BQS-Alexa 488	

FIG. 6B

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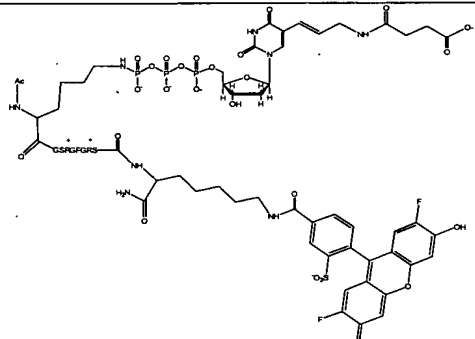
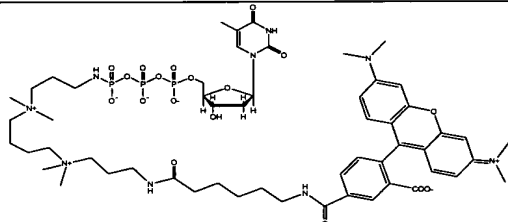
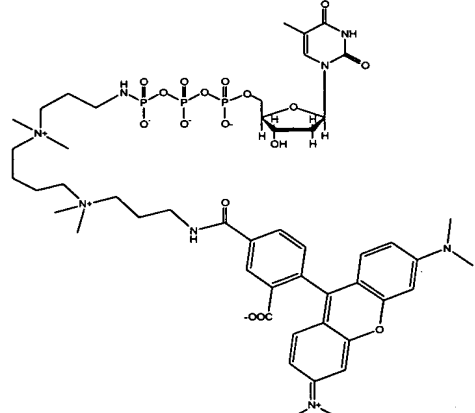
55	N = -1 F = +1	Suc-U-peptide+2-Oregon 500	
56	N = 0 F = 2	T-BQS-TAMRA X	
57	N = 0 F = 2	T-BQS-TAMRA	

FIG. 6C

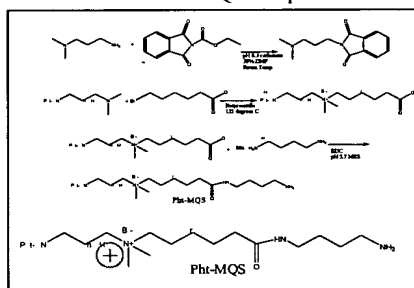
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58	N = +1 F = +1	AA-U-BQS-Oregon 500	
59	N = 0 F = +1	G-BQS-Oregon 500	
60	N = +2 F = +1	PAA-C-BQS-Oregon 500	

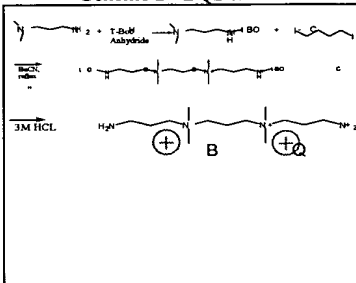
FIG. 6D

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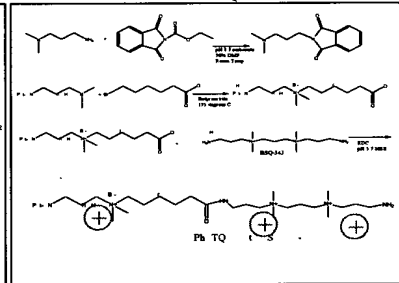
Scheme 1 - MQS component



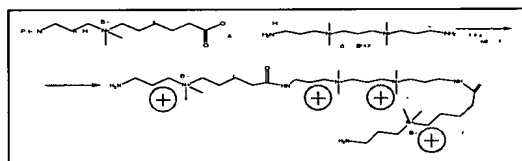
Scheme 2 - BQS linker



Scheme 3 - TQS linker

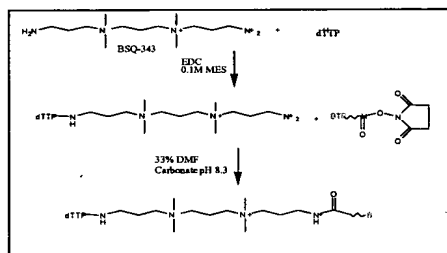


Scheme 4 - TetQS linker



Scheme 5 - Protect AA-dUTP

Use same chemistry as in Scheme 1, except the amine is the aminoallyl group of AA-dUTP. Show that deprotection can be accomplished in 1M NaOH, room temp, 2 hours, without degrading triphosphates.

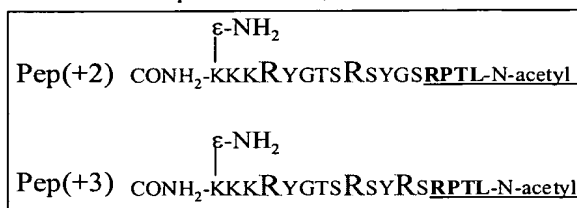


Scheme 6 - Coupling nucleotide, linker and dye (BTR is BodipyTR dye shown as the succinimide ester). We use this chemistry routinely to make γ -dNTPs (e.g, see cpd of Fig 13A).

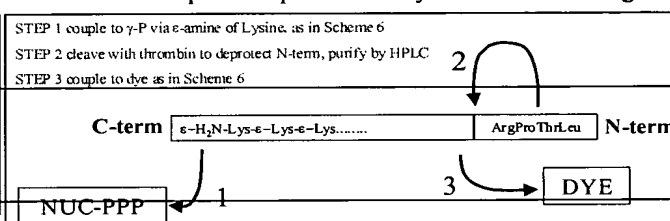
FIG. 6E

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Scheme 7 - Peptide linkers (shown in C-to-N direction)

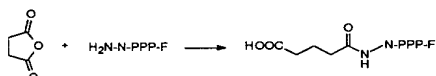


Scheme 8 - Peptide Deprotection By Thrombin Cleavage

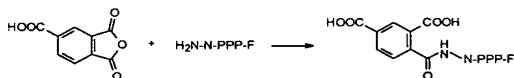


Scheme 9 - Add carboxylate to aminoallyl-dUTP

One Negative Charge:



Two Negative Charges:



Scheme 10 - γ -dNTP With Carboxylated Base

1. NH_2 -dU-PPP + Pht (of Scheme 1) \longrightarrow
2. Pht-NH-dU-PPP + ϵ NH₂-KKK-pep-RPTL \longrightarrow
3. Pht-NH-dU-PPP-KKK-pep-RPTL + 1M NaOH \longrightarrow
4. NH_2 -dU-PPP-KKK-pep-RPTL + anhydride (of Scheme 9) \longrightarrow
5. (COO^-) -dU-PPP-KKK-pep-RPTL + thrombin \longrightarrow
6. (COO^-) -dU-PPP-KKK-pep-NH₂ + SE-Dye \longrightarrow
7. (COO^-) -dU-PPP-KKK-pep-NH-Dye

FIG. 6F

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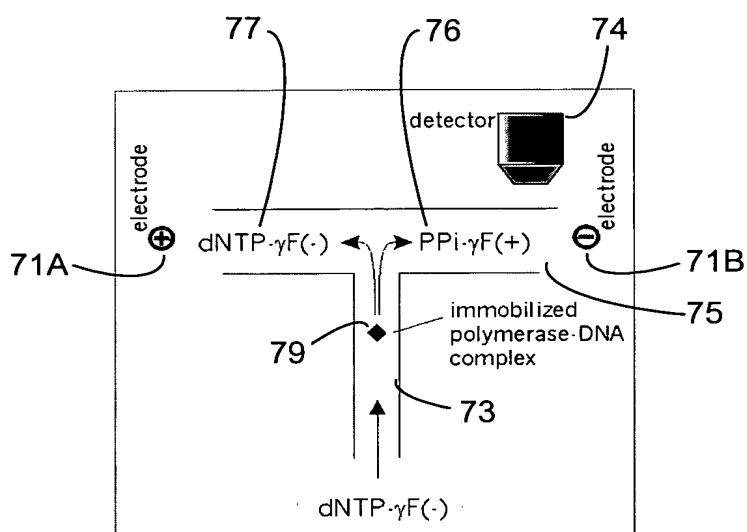


FIG. 7

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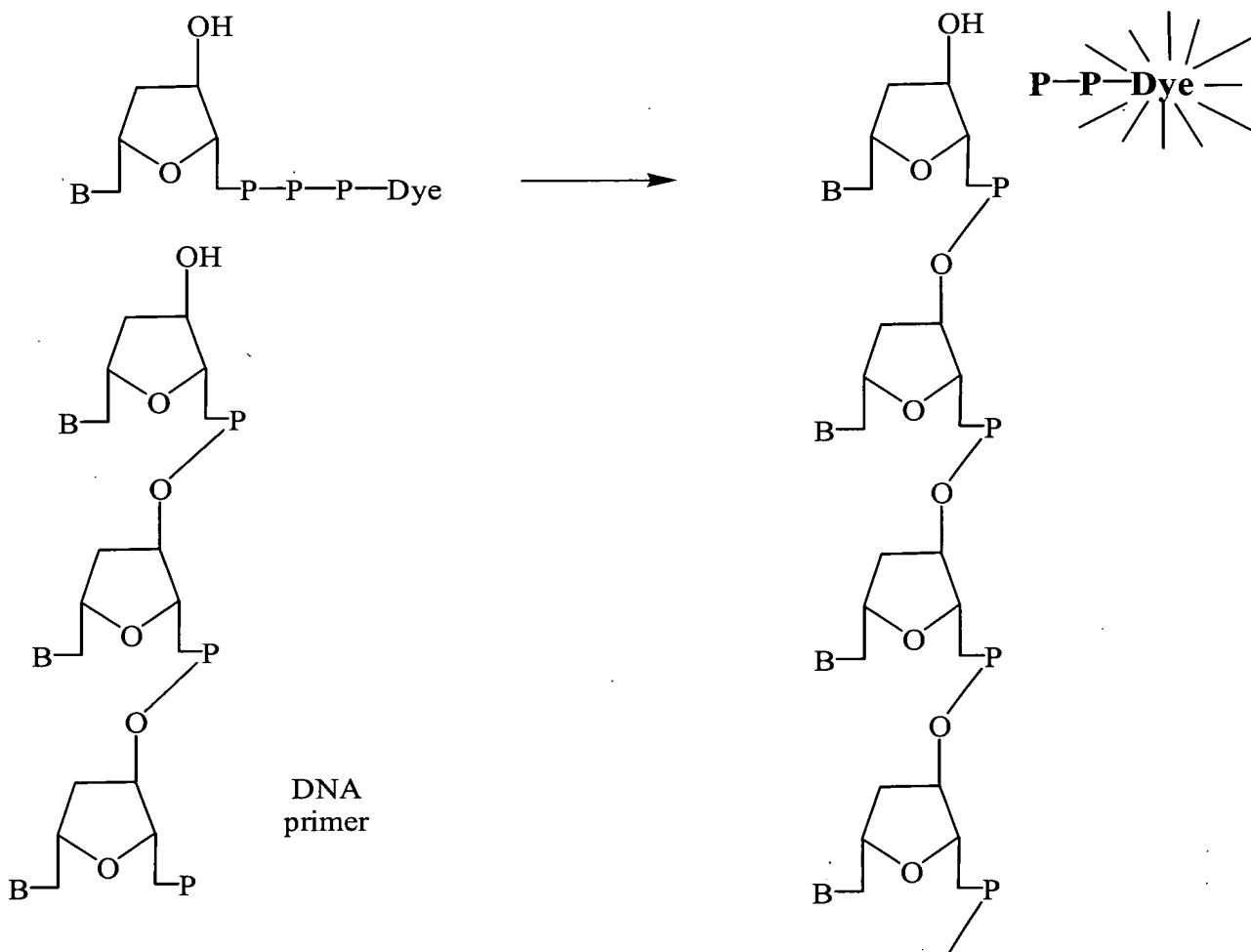


FIG. 8

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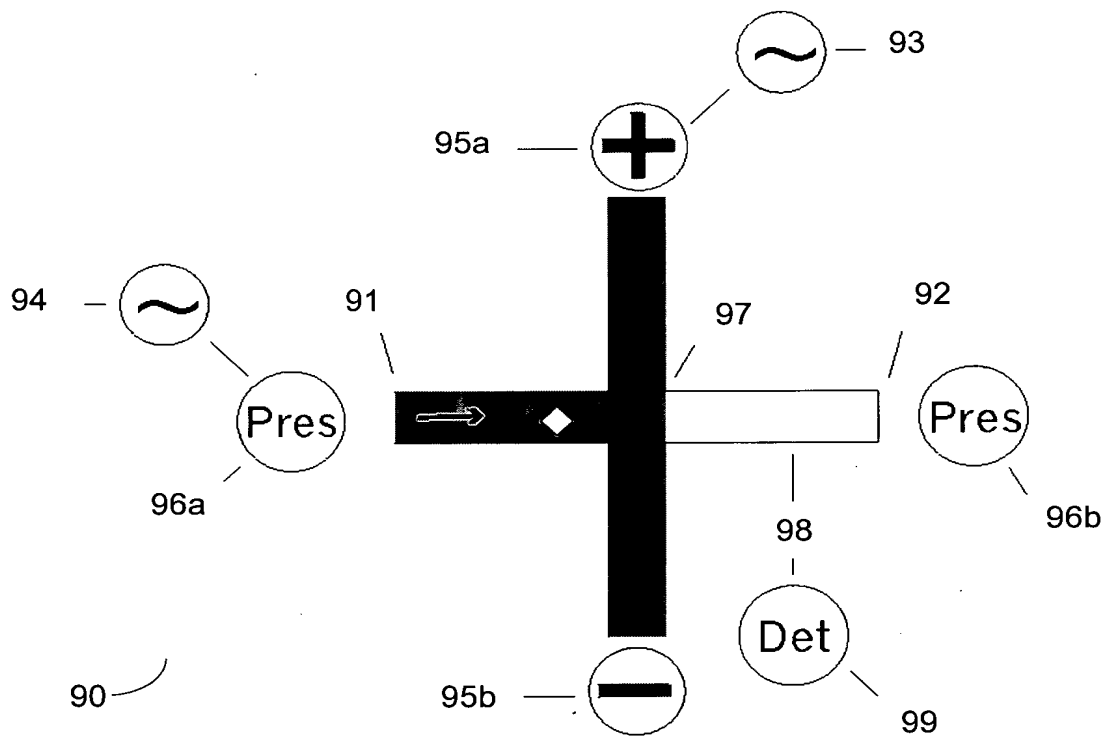


FIG. 9

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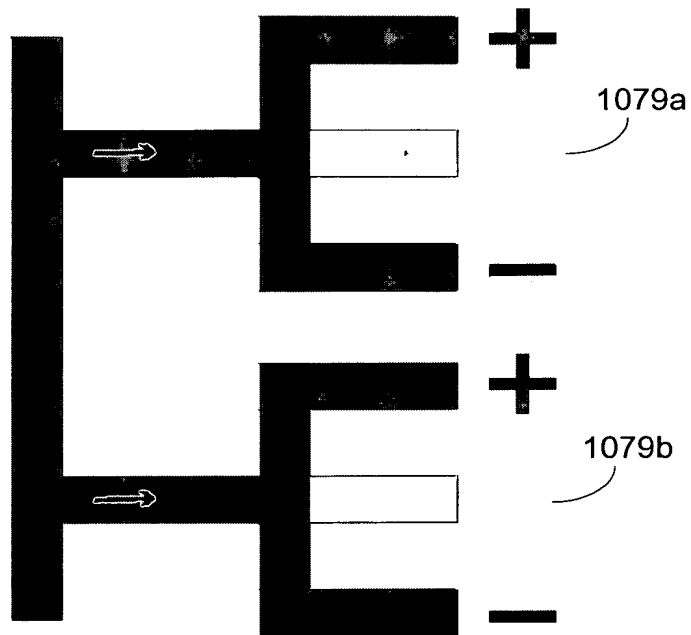
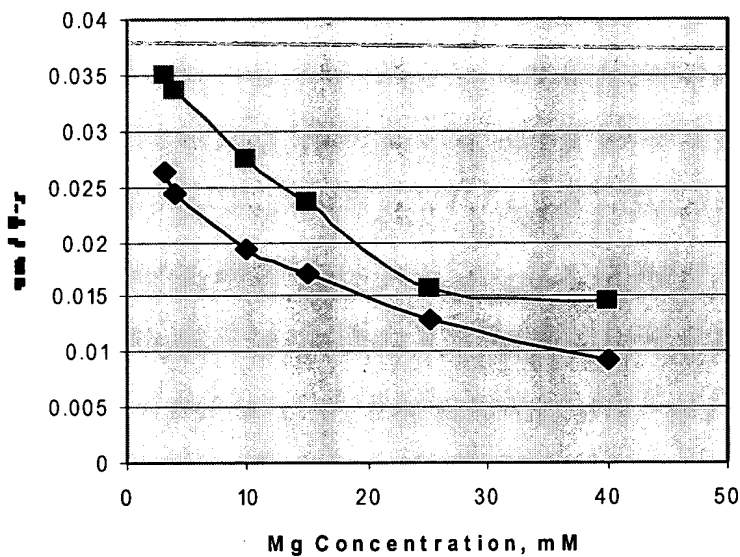


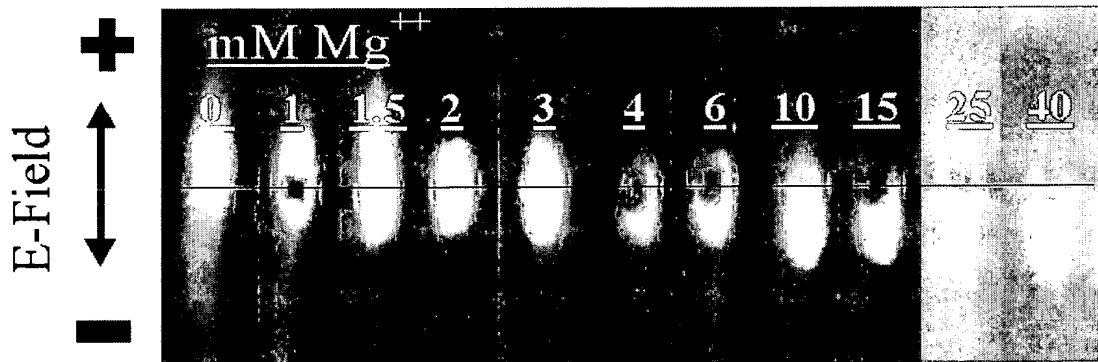
FIG. 10

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Mg⁺⁺ Controls Electrophoretic Mobility of Nucleotides



A



B

FIG. 11

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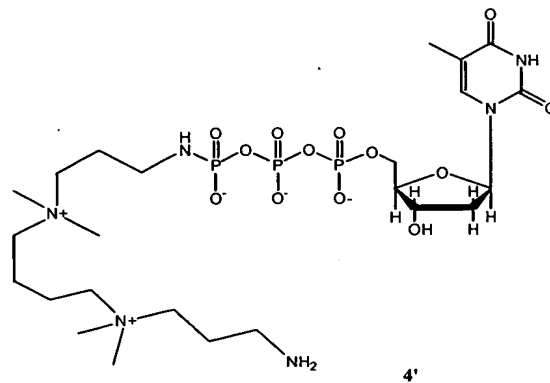
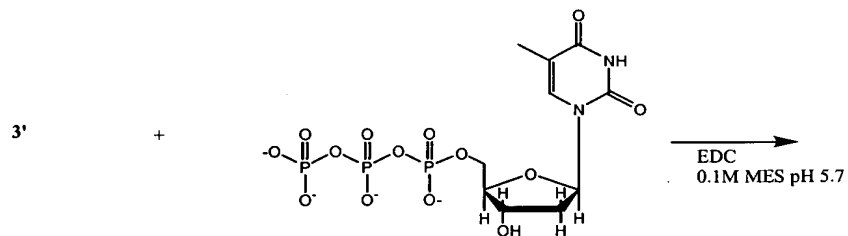
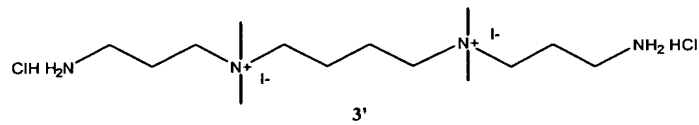
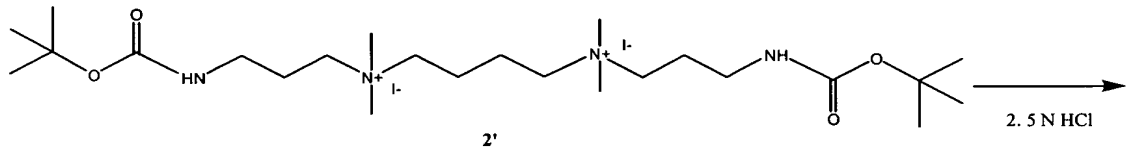
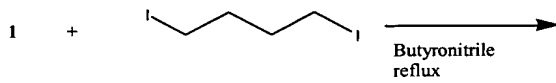
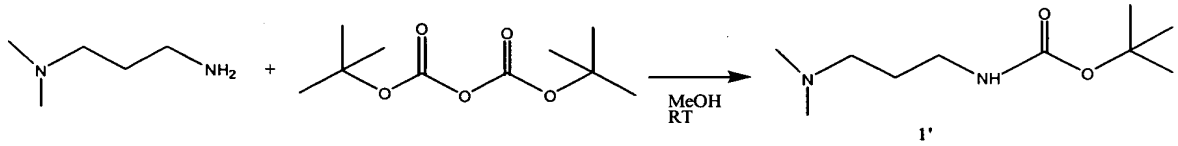


FIG. 12

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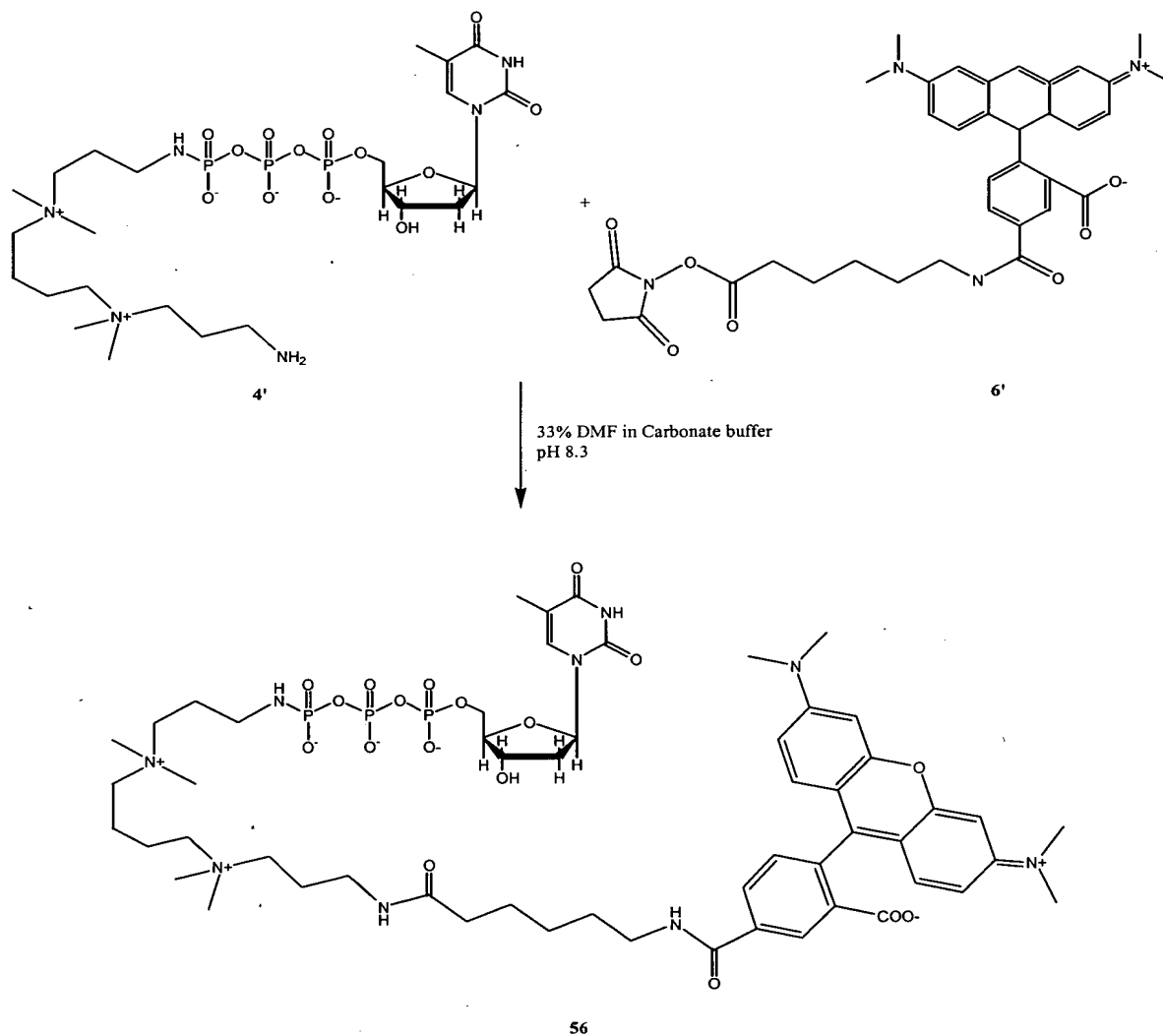


FIG. 12 (continued)